

CLAIMS

1. A polypeptide-polymer conjugate having
 - a) one or more additional polymeric molecules coupled to the polypeptide, having been modified in a manner to increase the number of attachment groups on the surface of the polypeptide, in comparison to the number of attachment groups available on the corresponding parent polypeptide, and/or
 - b) one or more fewer polymeric molecules coupled to the polypeptide, having been modified in a manner to decrease the number of attachment groups at or close to the functional site(s) of the polypeptide, in comparison to the number of attachment groups available on the corresponding parent polypeptide.
- 15 2. The conjugate of claim 1, having 1 to 25, preferably 1 to 10 additional polymeric molecules coupled to the surface of the polypeptide in comparison to the number of polymeric molecules of a conjugate prepared from the corresponding parent enzyme.
- 20 3. The conjugate of claim 1 or 2, wherein the additional attachment group(s) is(are) amino groups in the form of lysine residues(s), or carboxylic groups in the form of aspartic acid or glutamic acid residues.
- 25 4. The conjugate of any of claims 1 to 3, wherein the additional attachment group(s) is(are) prepared by a conservative substitution of an amino acid residue, such as an arginine to lysine substitution.
- 30 5. The conjugate of any of claims 1 to 3, wherein the additional attachment group(s) is(are) prepared by a conservative substitution of an amino acid, such as an asparagine to aspartate/glutamate or a glutamine to aspartate/glutamate substitution.
- 35 6. The conjugate of any of claims 1 to 5, wherein the added attachment group is located more than 5 Angstroms, preferably 8 Angstroms, especially 10 Angstroms from the functional site.
7. The conjugate of claim 1, having 1 to 25 preferably 1 to 10 fewer polymeric molecules coupled at or close to the functional site of the

polypeptide in comparison to the number of polymeric molecules of a conjugate prepared on the basis of the corresponding parent polypeptide.

8. The conjugate of claim 7, wherein the removed attachment group(s) 5 is(are) amino groups in the form of lysine residues(s), or carboxylic groups in the form of aspartic acid or glutamic acid residues.

9. The conjugate of claim 7 or 8, wherein the removed attachment group(s) is(are) prepared by a conservative substitution of an amino 10 group, such as lysine to arginine substitution.

10. The conjugate of claim 7 or 8, wherein the removed attachment group(s) is(are) prepared by a conservative substitution of a carboxylic group, such as an aspartate/glutamate to asparagine or 15 aspartate/glutamate to glutamine substitution.

11. The conjugate of any of claims 1 to 10, wherein the removed attachment group is located within 5 Angstroms, preferably 8 Angstroms, especially 10 Angstroms from the functional site.

20

12. The conjugate of any of claims 1 to 11, wherein the attachment groups are broadly spread.

13. The conjugates of any of claims 1 to 12, wherein the parent 25 polypeptide moiety of the conjugate has a molecular weight from 1 to 100 kDa, preferred 15 to 100 kDa.

14. The conjugate of claim 13, wherein the parent polypeptide moiety of the conjugate has a molecular weight of from 1 to 35 kDa.

30

15. The conjugates of claim 14, wherein the parent polypeptide is an enzyme selected from the group of oxidoreductases, including laccases and superoxide dismutase (SOD); hydrolases, including proteases, especially subtilisins, and lipolytic enzymes; transferases, including 35 transglutaminases (TGases); isomerases, including Protein disulfide Isomerases (PDI).

16. The conjugate of claim 15, wherein the parent enzyme is PD498, SAVINASE®, BPN¹, Proteinase K, Proteinase R, Subtilisin DY, Lion Y,

RENNILASE®, JA16, ALCALASE® or a *Humicola lanuginosa* lipase, such as LIPOLASE®.

17. The conjugate of claim 16, wherein the enzyme moiety of the
5 conjugate is a PD498 variant with one or more of the following substitutions: R51K, R62K, R121K, R169K, R250K, R28K, R190K, P6K, Y7K, S9K, A10K, Y11K, Q12K, D43K, Y44K, N45K, N65K, G87K, I88K, N209K, A211K, N216K, N217K, G218K, Y219K, S220K, Y221K, G262K.

10 18. The conjugate of claim 17, with one of the following mutations:
R28K+R62K, R28K+R169K, R62K + R169K, R28K+R69K+R169K.

19. The conjugate of claim 16, wherein the enzyme moiety of the conjugate is a SAVINASE® variant with one or more of the following substitutions: R10K, R19K, R45K, R145K, R170K, R186K, R247K, K94R, P5K, P14K, T22K, T38K, H39K, P40K, L42K, L75K, N76K, L82K, P86K, S103K, V104K, S105K, A108K, A133K, T134K, L135K, Q137K, N140K, N173K, N204K, Q206K, G211K, S212K, T213K, A215K, S216K, N269K.

20 20. The conjugate of claim 16, wherein the enzyme moiety of the conjugate is a *Humicola lanuginosa* lipase variant with one or more of the following substitutions:

R133K, R139K, R160K, R179K, R209K, R118K, R125K, A18K, G31K, T32K, N33K, G38K, A40K, D48K, T50K, E56K, D57K, S58K, G59K, V60K, G61K,
25 D62K, T64K, L78K, E87K, N88K, G91K, N92K, L93K, S105K, G106K, V120K, P136K, G225K, L227K, V228K, P229K, P250K, D254K, F262K.

21. The conjugate of claim 20 with the following mutations E87K+D254K.

30 22. The conjugate of any of claims 1 to 21, wherein the polymeric molecules coupled to the polypeptide have a molecular weight from 1 to 60 kDa, especially 1-35 kDa, especially 3 to 25 kDa.

23. The conjugate of claim 22, wherein the polymeric molecule is
35 selected from the group comprising a natural or synthetic homo- and heteropolymers, selected from the group of the synthetic polymeric molecules including Branched PEGs, poly-vinyl alcohol (PVA), poly-carboxyl acids, poly-(vinylpyrrolidone) and poly-D,L-amino acids, or natural occurring polymeric molecules including dextrans, including

carboxymethyl-dextrans, and celluloses such as methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, and hydrolysates of chitosan, starches, such as hydroxyethyl-starches, hydroxypropyl-starches, glycogen, agarose, guar 5 gum, inulin, pullulans, xanthan gums, carrageenin, pectin and alginic acid.

24. A method for preparing improved polypeptide-polymer conjugates comprising the steps of:

- 10 a) identifying amino acid residues located on the surface of the 3D structure of the parent polypeptide in question,
- b) selecting target amino acid residues on the surface of said 3D structure of said parent polypeptide to be mutated,
- c) i) substituting or inserting one or more amino acid residues selected in step b) with an amino acid residue having a suitable attachment group, and/or ii) substituting or deleting one or more amino acid residues selected in step b) at or close to the functional site,
- d) coupling polymeric molecules to the mutated polypeptide.

20 25. The method of claim 24, wherein the identification of amino acid residues located on the surface on the polypeptide referred to in step a) are performed by a computer program analyzing the 3D structure of the parent polypeptide in question.

25 26. The method of claim 24, wherein step b) comprises selecting arginine or lysine residues on the surface of the parent polypeptide.

27. The method of claim 24, wherein one or more arginine residues identified in step b) is(are) substituted with a lysine residue(s) in 30 step c).

28. The method of claims 27, wherein the substituted arginine residues have a distance of more than 5 Angstroms, preferably 8 Angstroms, especially 10 Angstroms from the functional site.

35

29. The method of any of claims 24 to 28, wherein the polypeptide prepared in step c) is coupled to polymeric molecules.

30. Use of the conjugate of any of claims 1 to 23 for reducing the allergenicity of industrial products.

31. Use of the conjugate of any of claims 1 to 23 for reducing the 5 immunogenicity of pharmaceuticals.

32. A composition comprising a conjugate of any of claims 1 to 23 and further comprising ingredients used in industrial products.

10 33. The composition of claim 32, wherein the industrial product is a detergent, such as a laundry, dish wash or hard surface cleaning product, or a food or feed product.

34. The composition of claim 32, comprising a conjugate of any of 15 claims 1 to 22 and further ingredients used in skin care products.

35. A composition comprising a conjugate of any of claims 1 to 23 and further comprising ingredients used in pharmaceuticals.

20 36. A conjugate comprising a lipase moiety conjugated to one or more polymers, wherein the lipase moiety is a *Humicola lanuginosa* lipase which comprises one or more of the following substitutions:
A18K, G31K, T32K, N33K, G38K, A40K, D48K, T50K, E56K, D57K, S58K, G59K, V60K, G61K, D62K, T64K, L78K, N88K, G91K, N92K, L93K, S105K, G106K,
25 R118K, V120K, R125K, R133K, P136K, R139K, R160K, R179K, R209K, G225K, L227K, V228K, P229K, P250K and F262K.

37. The conjugate of claim 36, wherein the *Humicola lanuginosa* lipase has an amino acid sequence of SEQ ID NO: 6.

30 38. The conjugate of claim 36, wherein the polymer(s) have a molecular weight from 1 to 60 kDa.

39. The conjugate of claim 36, wherein the polymer(s) are natural or
35 synthetic homo- or heteropolymers.

40. The conjugate of claim 36, wherein the polymer(s) are selected from the group consisting of polyols, polyamines, polycarboxyl acids and polymers comprising a hydroxyl group and an amine group.

41. The conjugate of claim 36, wherein the polymer(s) are selected from the group consisting of polyalkylene oxides (PAO), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs,
5 polyvinyl alcohols (PVA), poly-carboxylates, polyvinylpyrrolidones, poly-D,L-amino acids, polyethylene-co-maleic acid anhydride, polystyrene-co-malic acid anhydrides, dextrans, heparins, homologous albumins, celluloses, hydrolysates of chitosan, starches, glycogen, agaroses and derivatives thereof, guar gum, pullulan, inulin, xanthan gum,
10 carrageenin, pectin, alginic acid hydrolysates and bio-polymers.

42. The conjugate of claim 36, wherein the polymer(s) are polyalkylene glycols (PAG) or methoxypolyethylene glycols (mPEG).

15 43. The conjugate of claim 36, wherein the polymer(s) are selected from the group consisting of polyethylene glycols (PEG), polypropylene glycols and carboxymethyl-dextrans.

44. The conjugate of claim 36, wherein the polymer(s) are selected
20 from the group consisting of methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose carboxyethylcellulose and hydroxypropylcellulose.

45. The conjugate of claim 36, wherein the polymer(s) are
25 hydroxyethyl-starches or hydroxypropyl-starches.

46. The conjugate of claim 36, wherein the polymer(s) are methoxypolyethylene glycols (mPEG).

30 47. A detergent composition comprising a conjugate of claim 36 and a surfactant.

48. A skin care composition, comprising a conjugate of claim 36 and ingredients used in skin care products.

35 49. A pharmaceutical composition comprising a conjugate of claim 36 and further comprising ingredients used in pharmaceuticals.